drug should be limited to hatchery or laboratory use.

[40 FR 13881, Mar. 27, 1975, as amended at 49 FR 5748, Feb. 15, 1984; 51 FR 11439, Apr. 3, 1986]

PART 556—TOLERANCES FOR RESIDUES OF NEW ANIMAL DRUGS IN FOOD

Subpart A—General Provisions

Sec.

Subpart B—Specific Tolerances for Residues of New Animal Drugs

AUTHORITY: Secs. 402, 512, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 342, 360b, 371).

SOURCE: 40 FR 13942, Mar. 27, 1975, unless otherwise noted.

Subpart A—General Provisions

§ 556.1 General considerations; tolerances for residues of new animal drugs in food.

(a) Tolerances established in this part are based upon residues of drugs in edible products of food-producing animals treated with such drugs. Consideration of an appropriate tolerance for a drug shall result in a conclusion either that:

(1) Finite residues will be present in the edible products—in which case a finite tolerance is required; or

(2) It is not possible to determine whether finite residues will be incurred but there is reasonable expectation that they may be present—in which case a tolerance for negligible residue is required; or

(3) The drug induces cancer when ingested by man or animal or, after tests which are appropriate for the evaluation of the safety of such drug, has been shown to induce cancer in man or animal; however, such drug will not adversely affect the animals for which it is intended, and no residue of such drug will be found by prescribed methods of analysis in any edible portion of such animals after slaughter or in any food yielded by or derived from the living animal—in which case the accepted method of analysis shall be published or cited, if previously published and available elsewhere, in this part; or

(4) It may or may not be possible to determine whether finite residues will be incurred but there is no reasonable expectation that they may be present—in which case the establishment of a tolerance is not required; or

(5) The drug is such that it may be metabolized and/or assimilated in such form that any possible residue would be indistinguishable from normal tissue constituents—in which case the establishment of a tolerance is not required.

(b) No tolerance established pursuant to paragraph (a)(1) of this section will be set at any level higher than that reflected by the permitted use of the drug.

(c) Any tolerance required pursuant to this section will, in addition to the toxicological considerations, be conditioned on the availability of a practicable analytical method to determine the quantity of residue. Such method must be sensitive to and reliable at the established tolerance level or, in certain instances, may be sensitive at a higher level where such level is also deemed satisfactory and safe in light of the toxicity of the drug residue and of the unlikelihood of such residue's exceeding the tolerance.

Subpart B—Specific Tolerances for Residues of New Animal Drugs

§ 556.20 2-Acetylamino-5-nitrothiazole.

A tolerance of 0.1 part per million is established for negligible residues of 2-acetylamino-5-nitrothiazole in the edible tissues of turkeys.

§ 556.30 Aklomide.

Tolerances are established for combined residues of aklomide (2-chloro-4-nitrobenzamide) and its metabolite (4-amino-2-chlorobenzamide) in uncooked edible tissues of chickens as follows:

(a) 4.5 parts per million in liver and muscle.

(b) 3 parts per million in skin with fat.

§ 556.34 Albendazole.

Tolerances are established for residues of albendazole in uncooked edible tissues as follows:

(a) *Cattle.* The tolerance for the 2-aminosulfone metabolite (marker residue) in cattle liver (target tissue) is 0.2 part per million. The tolerance refers to the concentration of marker residue in the target tissue used to monitor for total drug residues in the target animals.

(b) *Sheep.* The tolerance for the 2-aminosulfone metabolite (marker residue) in sheep liver (target tissue) is 0.25 part per million.

[59 FR 65711, Dec. 21, 1994]

§ 556.38 Amoxicillin.

A tolerance of 0.01 part per million is established for negligible residues of amoxicillin in milk and in the uncooked edible tissues of cattle.

[49 FR 45422, Nov. 16, 1984]

§ 556.40 Ampicillin.

A tolerance of 0.01 p/m is established for negligible residues of ampicillin in the uncooked edible tissues of swine and cattle and in milk.

§ 556.50 Amprolium.

Tolerances are established as follows for residues of amprolium (1-(4-amino-2-*n*-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride):

(a) In the edible tissues and in eggs of chickens and turkeys:

(1) 1 part per million in uncooked liver and kidney.

(2) 0.5 part per million in uncooked muscle tissue.

(3) In eggs:

(i) 8 parts per million in egg yolks.

(ii) 4 parts per million in whole eggs.

(b) In the edible tissues of calves:

(1) 2.0 parts per million in uncooked fat.

(2) 0.5 part per million in uncooked muscle tissue, liver, and kidney.

(c) In the edible tissues of pheasants:

(1) 1 part per million in uncooked liver.

(2) 0.5 part per million in uncooked muscle.

[40 FR 13942, Mar. 27, 1975, as amended at 50 FR 18472, May 1, 1985]

§ 556.52 Apramycin.

Tolerances of 0.1 part per million are established for total residues of apramycin in uncooked swine muscle, 0.3 part per million for liver, and 0.4 part per million for kidney and fat. A drug residue assay measuring parent apramycin (the marker residue) in the target tissue, kidney, serves to monitor the total residue in edible tissues. A marker residue concentration of 0.1 part per million in kidney corresponds to 0.4 part per million total residue in this target tissue.

[47 FR l5771, Apr. 13, 1982]

§ 556.60 Arsenic.

Tolerances for total residues of combined arsenic (calculated as As) in food are established as follows:

(a) In edible tissues and in eggs of chickens and turkeys:

(1) 0.5 part per million in uncooked muscle tissue.

(2) 2 parts per million in uncooked edible by-products.

(3) 0.5 part per million in eggs.

(b) In edible tissues of swine:

(1) 2 parts per million in uncooked liver and kidney.

(2) 0.5 part per million in uncooked muscle tissue and by–products other than liver and kidney.

§ 556.70 Bacitracin.

Tolerances for residues of bacitracin from zinc bacitracin or bacitracin methylene disalicylate are established at 0.5 part per million (0.02 unit per gram), negligible residue, in uncooked edible tissues of cattle, swine, chickens, turkeys, pheasants, and quail, and in milk and eggs.

[42 FR 18614, Apr. 8, 1977]

§ 556.90 Buquinolate.

Tolerances are established for residues of buquinolate as follows:

(a) In edible tissues of chickens:

(1) 0.4 part per million in uncooked liver, kidney, and skin with fat.

(2) 0.1 part per million in uncooked muscle.

(b) In eggs:

(1) 0.5 part per million in uncooked yolk.

(2) 0.2 part per million in uncooked whole eggs.

§ 556.100 Carbadox.

No residues of carbadox (Methyl 3-(2-quinoxalinylmethylene) carbazate-N^1, N^4-dioxide) and its metabolite (quinoxaline-2-carboxylic acid) are found in the uncooked edible tissues of swine as determined by the following method of analysis:

I. REAGENTS

A. Benzene—Distilled-in-Glass grade, Burdick and Jackson Laboratories or equivalent.

B. Ethyl acetate—Distilled-in-Glass grade, Burdick and Jackson Laboratories or equivalent.

C. n-Hexane—Distilled-in-Glass grade, Burdick and Jackson Laboratories or equivalent.

D. 1-Propanol—reagent grade, dried over molecular sieve pellets (5A).

E. Citric acid monohydrate—U.S.P., Pfizer, Inc., or equivalent.

F. Potassium hydroxide—pellets, reagent grade.

G. Sodium hydroxide—pellets, reagent grade.

H. Hydrochloric acid—reagent, A.C.S.

I. Sulfuric acid—reagent, A.C.S.

J. Sodium sulfate—anhydrous, reagent grade.

K. Quinoxaline-2-carboxylic acid—Pfizer, Inc., or equivalent.

L. Propyl quinoxaline-2-carboxylate—Pfizer, Inc., or equivalent.

M. Acridine—practical grade; Matheson Coleman and Bell or equivalent.

II. SOLUTIONS

A. 1*M* Citric acid.

B. 5*M* Sodium hydroxide.

C. 3*M* Potassium hydroxide.

D. 0.5*M* Citric acid buffer. Adjust the pH of 100 milliliters of 1*M* citric acid to pH 6.0 with 5*M* sodium hydroxide (approximately 55 milliliters), using a previously calibrated pH meter. Adjust the final volume to 200 milliliters with distilled water. Before making the final pH adjustment, cool the buffer to room temperature.

E. 1-Propanol-sulfuric acid reagent (97:3). Dilute 3 milliliters of concentrated sulfuric acid to 100 milliliters with dried, filtered, and cooled 1-propanol.

F. Acridine solution. Dissolve 1 milligram of acridine in 100 milliliters of benzene.

G. Quinoxaline-2-carboxylic acid solutions:

1. *Stock solution A.* Dissolve 1.25 milligram of quinoxaline-2-carboxylic acid in enough 1-propanol to make 100.0 milliliters (concentration 12.5 micrograms per milliliter).

2. *Stock solution B.* Dilute 1.0 milliliter of stock solution A to 100.0 milliliters with 1-propanol-sulfuric acid reagent (concentration 0.125 microgram per milliliter).

3. *Working standard solution C.* Dilute a 2.0 milliliter aliquot of stock solution B to 10.0 milliliters with 1-propanol-sulfuric acid reagent (concentration 25.0 nanograms per milliliter).

4. *Working standard solution D.* Dilute a 3.0 milliliter aliquot of stock solution B to 10.0 milliliters with 1-propanol-sulfuric acid reagent (concentration 37.5 nanograms per milliliter).

5. *Working standard solution E.* Dilute a 4.0 milliliter aliquot of stock solution B to 10.0 milliliters with 1-propanol-sulfuric acid reagent (concentration 50.0 nanograms per milliliter).

6. *Fortification solution.* Dilute 3.0 milliliters stock solution A to 250 milliliters with distilled water (concentration 150 nanograms per milliliter).

7. *Propyl quinoxaline-2-carboxylate solution.* Dissolve 1.00 milligram of propyl quinoxaline-2-carboxylate in enough ethyl acetate to make 10 milliliters (concentration 100 micrograms per milliliter).

III. APPARATUS

A. Column, glass-tapered at one end, 0.9 centimeters x 21.5 centimeters, prepared from a 10-milliliter serological pipette.

B. Centrifuge tubes, heavy duty—50-milliliter graduated (60-milliliter capacity), equipped with glass stoppers, R. C. Ewald, Inc., or equivalent.

C. Centrifuge tubes—50-milliliter graduated, equipped with glass stoppers.

D. Volumetric flasks—5 10 100 and 250-milliliter capacity, glass stoppered.

E. Pipettes, automatic transfer—10 15 and 25-milliliter delivery volume.

F. Pipettes, measuring—0.1 and 0.5 milliliter delivery volume.

G. Pipettes, volumetric—1 2 3 4 and 5-milliliter delivery volume.

H. Pipette, serological—10 milliliter delivery volume.

I. Pipettes—Pasteur, disposable.

J. Propipette bulb.

K. Syringe—10 microliter capacity, Hamilton or equivalent.

L. Crystallizing dish—190 millimeter (diameter) x 100 millimeter (height), for oil bath.

M. Test tube rack.

N. Test tube mixer—Vortex mixer or equivalent.

O. Lab jack—Cenco or equivalent.

P. Thermo-stir hotplate.

Q. Magnetic stirrer bar (teflon).

R. Thermometer—centigrade, 0° to 150° C. range.

S. Knife (for cutting frozen tissue).

T. Ultraviolet light—254 nanometers and 366 nanometers.

U. Scalpel.

V. Torsion balance—style RX–1, class A, Torsion Balance Co., or equivalent.

W. Cahn electrobalance—Cahn Model C–2 or equivalent.

X. Centrifuge—International, size 2, model K, or equivalent.

Y. Rotary evaporator equipped either with a water aspirator or with a vacuum pump and condenser.

Z. Alkacid test paper.

AA. Glassine paper.

BB. Glasswool.

CC. Flask—round bottom, 29/42 ST, 250 milliliters.

DD. Flask—round bottom, 19/22 ST, 65 milliliters.

EE. Funnel—burette.

FF. Hair dryer.

GG. pH meter.

HH. Tray—instrument, stainless steel.

II. Water bath.

JJ. Precoated thin layer plates—20 x 20 centimeters; 250 micron thickness, Silica gel GF, E. Merck, Darmstadt; distributed by Brinkmann Instruments Inc., Westbury, NY 11590 or equivalent.

KK. Desaga multiplate developing tanks for five 20 x 20 centimeters plates-distributed by Brinkmann Instruments Inc., or equivalent.

LL. Gas-liquid chromatograph—Micro Tek 220 model instrument (or equivalent) equipped with a Ni^{63} electron affinity pulsed detector and a 0–1 MV recorder. Conditions and operating parameters for the gas-liquid chromatograph are: Isothermal column temperature, 175° C.; inlet heater, 270° C.; EC detector temperature, 275° C.; argonmethane

(95:5) flowrate, 100 milliliters per minute (40 pounds per square inch); chart speed, ½ inch per minute, attentuation, 10 x 64. Electrometer pulse parameters: RF mode; voltage output, 55; pulse rate, 270 microseconds; pulse width, 3.0 microseconds.

A glass sleeve injection port liner is installed for off-column injections.

MM. Packing—3 percent OV–17 on Gas Chrom Q, 60–80 mesh, Applied Sciences Laboratories, Inc. or equivalent.

NN. Column—pyrex glass, U-tube, 6 feet (length) x 4 millimeters (inside diameter). Condition the packed column at 280° C. for at least 72 hours with argon-methane (95:5) flow, detached from the detector input.

OO. Septum—high temperature type (HT–13), Applied Sciences Laboratories, Inc. or equivalent.

PP. Detector-Nickel[63] electron capture. The voltage current profile for this detector should plateau at 30 volts or less in the DC mode when a stream of nitrogen gas is passed through the column and the electron capture detector.

IV. PROCEDURE

A. DISSOLUTION AND HYDROLYSIS STEP

Transfer 5 grams of swine tissue (freshly sliced from frozen tissue) to a 50-milliliter centrifuge tube. Add 10 milliliters of 3*M* potassium hydroxide, stopper, and place in a 100° C. silicone oil bath for 1 hour.

NOTE: The level of the silicone oil bath should exceed that of the tissue sample. Stopper the tubes lightly in order to allow the digestion mixture to ''breath''. To determine the recovery of quinoxaline-2-carboxylic acid in swine tissue at the 30 p.p.b. level, fortify 5 grams of sample with 1 milliliter of fortification solution (concentration 150 nanograms per milliliter).

B. EXTRACTION STEP

1. Cool the alkaline hydrolyzate in an ice bath and acidify to 1 pH 1 (deep red to alkacid test paper) with 4 milliliters of concentrated hydrochloric acid. Add 15 milliliters of ethyl acetate to the acidified hydrolyzate, stopper, and extract by shaking for 20 seconds. Centrifuge the mixture at 1,500 revolutions per minute for 5 minutes to clarify the ethyl acetate phase. Recover the ethyl acetate phase with a blowout pipette equipped with a propipette bulb, and transfer this extract to a 60-milliliter separatory funnel equipped with teflon stopcocks. Reextract the hydrolyzate with two additional 15-milliliter portions of ethyl acetate, and combine the organic extracts.

NOTE: Do not contaminate the ethyl acetate phase with interfacial material during these extractions. Quinoxaline-2-carboxylic acid is unstable in strongly acidic solutions. Continue to process these extracts through the benzene extraction and evaporation steps.

2. Add 5 milliliters of 0.5*M* citric acid buffer (pH 6.0) to the ethyl acetate extract, shake, and allow the lower phase to clarify for about 20 minutes. Collect the aqueous phase in a 50-milliliter glass-stoppered centrifuge tube. Reextract the ethyl acetate phase with an additional 5 milliliters of pH 6 buffer, wait for the aqueous phase to clarify, and combine the aqueous extracts. Acidify (pH 1) the aqueous extract with 2 milliliters of concentrated hydrochloric acid, stopper, and extract with 25 milliliters of benzene. Centrifuge to clarify the benzene layer and transfer the organic phase, using a blowout pipette equipped with a propipette bulb, to a 250-milliliter round bottom flask. Repeat the extraction and centrifugation steps three times. Combine the benzene extracts (about 100 milliliters) and evaporate to near-dryness, using a rotary evaporator equipped with a water aspirator and with a water bath set at 40° C.

NOTE: A rotary evaporator equipped with a vacuum pump and condenser may be used at this point. These residues may be stored overnight.

C. ESTERIFICATION STEP

Reconstitute the residue from the previous step by rinsing the walls of the round bottom flask with 2 x 2 milliliters of 1-propanol-sulfuric acid reagent; transfer each rinse with a disposable pipette to a 50-milliliter centrifuge tube. Stopper and heat the tube in a silicone oil bath at 90° C. for 1 hour. Cool the reaction mixture in an ice bath before proceeding to the following extraction step.

NOTE: Samples and standards may be stored overnight at room temperature in the propanol-sulfuric acid medium.

D. EXTRACTION OF THE ESTER DERIVATIVE

Add 10 milliliters of water and 15 milliliters of n-hexane to the esterification mixture. Extract and centrifuge to clarify the n-hexane phase. Transfer the n-hexane extract to a 65-milliliter round bottom flask; reextract the aqueous-propanol phase with two additional 15-milliliter portions of n-hexane. Centrifuge after each extraction and combine the n-hexane extracts. (NOTE: Avoid taking any of the aqueous phase in this extraction step; otherwise, the n-hexane extracts will have to be washed with 3 x 10 milliliters of water and dried over sodium sulfate.) Concentrate this solution to 0.5 milliliter, using a rotary evaporator equipped with a water aspirator and with a water bath set at 25° C. (NOTE: A rotary evaporator equipped with a vacuum pump and condenser may be used at this point.) Fortify this solution with 0.1 milliliter of acridine marker (1 milligram per 100 milliliters benzene).

NOTE: Do not store the n-hexane extracts of the propyl ester derivative overnight. Continue to process these solutions by the following thin-layer chromatography step E.

E. THIN-LAYER CHROMATOGRAPHY

1. Quantitatively transfer the concentrated n-hexane extract to the ''origin'' of a 20-centimeters x 20-centimeters silica gel thin-layer plate, using a disposable pipette. When pipetting this extract, streak it in a uniform band approximately 15 centimeters across and approximately 20 millimeters above the lower edge of the plate, making sure not to scratch or remove appreciable portions of adsorbent and avoiding application of the sample to the sides of the plate. The applied band should not diffuse or penetrate to the end of the silica gel layer, but should remain 10 millimeters above the lower edge of the silica gel layer. Rinse the round bottom flask (containing residual n-hexane) with three portions of approximately 0.25 milliliter each of ethyl acetate; transfer each portion with the same pipette and cover the same area of the plate as described above. Following each application of the extract and ethyl acetate washes, evaporate the solvent from the plate by directing a stream of cool air to the sample zone (''origin''). Prior to chromatographic development, place an edge (approximately 5 millimeters deep) of the thin-layer plate into a tray of ethyl acetate so that the solvent will rise through the applied sample zone to form it into a narrow band approximately 10 millimeters above the ''origin.'' Air dry this plate before chromatographic development.

2. Place the prepared plate in a chromatographic chamber lined with blotting paper and saturated with the benzene-ethyl acetate system (85:15). Develop the plate twice in this system, maintaining straight solvent fronts and allowing the solvent front to reach the top of the plate during each irrigation. Air dry the thin-layer plate for approximately 5 minutes between the first and second irrigations. Each irrigation takes approximately 75 minutes. Developed plates should not be stored overnight. Examine the developed plate under long wavelength (366 nanometers) ultraviolet light and locate the blue fluorescent band of acridine (R_f approximately 0.5). Mark out a 12-millimeters x 20-centimeters band of silica gel encompassing an area 5 millimeters above and 7 millimeters below the center of the acridine marker and extending from one side of the plate to the other.

NOTE: The relative mobilities of propyl quinoxaline-2-carboxylate and acridine must be checked in each laboratory to determine where a 12-milliliter x 20-centimeters zone of silica gel is to be excised in order to quantitatively recover the propyl ester derivative. This may be accomplished by mixing 0.1 milliliter of acridine solution (1 milligram per 100 milliliters) with 0.4 milliliter of propyl quinoxaline-2-carboxylate (100 micrograms per milliliter) and chromatographing this solution as directed above. Examine the developed plate under long wavelength (366 nanometers) ultraviolet light and locate the blue fluorescent band of acridine (R_f approximately 0.5). Examination of the plate under short wavelength (254 nanometers) ultraviolet light locates the blue absorbing band of propyl quinoxaline-2-carboxylate (R_f approximately 0.5).

3. Reduce the sample zone to a fine powder by making a series of horizontal cuts with a scalpel. Gently transfer this powder with the aid of a stainless steel spatula to glassine paper; pour this material into a burette funnel atop a small glass column packed with a glass wool plug. Elute the adsorbent with ethyl acetate (about 6 milliliters), and collect the eluate to mark in a 5-milliliter volumetric flask. Examine this eluate by gas-liquid chromatography.

NOTE: Contamination of thin-layer chromatographic plates can be checked by gas-liquid chromatographic examination of an eluate prepared by processing a blank plate as in paragraph 1 above, starting at the point: ''place an edge (approximately 5 millimeters deep) of the thin-layer plate into a tray of ethyl acetate * * *.'' If the plate is contaminated, examine alternate lots of precoated thin-layer plates.

F. STANDARD CURVE

Pipette 4-milliliter aliquots of quinoxaline-2-carboxylic acid working standard solutions C, D, and E, respectively, and 4-milliliter portions of 1-propanol-sulfuric acid reagent into 50-milliliter centrifuge tubes; stopper, react, extract, and concentrate as directed in the esterification and extraction steps described in subsections C and D above; however, omit the addition of acridine to the n-hexane concentrate and do not chromatograph it by thin-layer chromatography. Instead, reconstitute the n-hexane concentrate with ethyl acetate and quantitatively transfer this solution to a 5-milliliter volumetric flask to give working standard solutions C, D, and E. The final concentrations of working standard solutions C, D, and E, are 20, 30, and 40 nanograms per milliliter, respectively, and are equivalent to 20, 30, and 40 p.p.b., respectively.

G. GAS-LIQUID CHROMATOGRAPHY

Separately inject 4 microliters of each of the working standard solutions C, D, and E (prepared as described above (F)) into the gas-liquid chromatograph to determine the retention time of propyl quinoxaline-2-carboxylate and the relative response of the EC detector. Construct a standard curve by

plotting concentration (p.p.b.) versus peak height (millimeters).

(NOTE: The reagent blank must show no interfering gas-liquid chromatographic peaks.) The peak height of propyl quinoxaline-2-carboxylate at the 30-p.p.b. level (working standard solution D) should approximate 10 percent of full-scale deflection with a retention time of 5 minutes. Follow these injections with 4-microliter injections of the tissue eluates, allowing 20 minutes between injections to clear the instrument of background peaks.

Measure the peak heights of samples and determine their concentration (p.p.b.) by reference to the standard curve.

H. CALCULATIONS

From the standard curve and the observed peak height of quinoxaline-2-carboxylic acid in the sample, determine its concentration (p.p.b.).

§ 556.110 Carbomycin.

A tolerance of zero is established for residues of carbomycin in the uncooked edible tissues of chickens.

§ 556.113 Ceftiofur.

Cattle, swine, and poultry: A tolerance for residues of ceftiofur in edible tissue is not required.

[57 FR 41862, Sept. 14, 1992]

§ 556.115 Cephapirin.

A tolerance of 0.02 parts per million (ppm) is established for residues of cephapirin in the milk and 0.1 ppm in the uncooked edible tissues of dairy cattle.

[40 FR 57454, Dec. 10, 1975]

§ 556.120 Chlorhexidine.

A tolerance of zero is established for residues of chlorhexidine in the uncooked edible tissues of calves.

§ 556.140 Chlorobutanol.

A tolerance of zero is established for residues of chlorobutanol in milk from dairy animals.

§ 556.150 Chlortetracycline.

Tolerances are established for residues of chlortetracycline in food as follows:

(a) In edible tissues and in eggs of chickens, turkeys, and ducks:

(1) 4 parts per million in uncooked kidney.

(2) 1 part per million in uncooked muscle, liver, fat, and skin.

(3) Zero in eggs.

(b) In edible tissues of swine:

(1) 4 parts per million in uncooked kidney.

(2) 2 parts per million in uncooked liver.

(3) 1 part per million in uncooked muscle.

(4) 0.2 part per million in uncooked fat.

(c) In edible tissues of calves:

(1) 4 parts per million in uncooked liver and kidney.

(2) 1 part per million in uncooked muscle and fat.

(d) In edible tissues of beef cattle and nonlactating dairy cows:

(1) 0.1 part per million in uncooked kidney, liver, and muscle.

(2) Zero in uncooked fat.

(e) Zero in milk.

(f) In edible tissues of sheep:

(1) 1 part per million in uncooked kidney.

(2) 0.5 part per million in uncooked liver.

(3) 0.1 part per million in uncooked muscle.

[40 FR 13942, Mar. 27, 1975, as amended at 49 FR 22634, May 31, 1984]

§ 556.160 Clopidol.

Tolerances for residues of clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) in food are established as follows:

(a) In cereal grains, vegetables, and fruits: 0.2 part per million.

(b) In chickens and turkeys:

(1) 15 parts per million in uncooked liver and kidney.

(2) 5 parts per million in uncooked muscle.

(c) In cattle, sheep, and goats:

(1) 3 parts per million in uncooked kidney.

(2) 1.5 parts per million in uncooked liver.

(3) 0.2 part per million in uncooked muscle.

(d) In swine: 0.2 part per million in uncooked edible tissues.

(e) In milk: 0.02 part per million (negligible residue).

§ 556.163 Clorsulon.

Tolerances are established for residues of clorsulon in cattle as follows:

(a) The tolerance for clorsulon (market residue) in kidney (target tissue) is 1.0 part per million. A marker residue of 1.0 part per million corresponds to a total residue of 3.0 parts per million in kidney.

(b) The safe concentrations for total clorsulon residues in uncooked edible cattle tissues are: muscle, 1.0 part per million; liver, 2.0 parts per million; kidney, 3.0 parts per million; and fat, 4.0 parts per million.

[50 FR 10221, Mar. 14, 1985]

§ 556.165 Cloxacillin.

A tolerance of 0.01 part per million is established for negligible residues of cloxacillin in the uncooked edible tissues of cattle and in milk.

[40 FR 28792, July 9, 1975]

§ 556.170 Decoquinate.

Tolerances for residues of decoquinate in food are established as follows in uncooked edible tissues of chickens, cattle, and goats at 2 parts per million in tissues other than skeletal muscle and 1 part per million in skeletal muscle.

[52 FR 43061, Nov. 9, 1987]

§ 556.180 Dichlorvos.

A tolerance of 0.1 part per million is established for negligible residues of dichlorvos (2,2-dichlorovinyl dimethyl phosphate) in the edible tissues of swine.

§ 556.200 Dihydrostreptomycin.

Tolerances are established for residues of dihydrostreptomycin in uncooked, edible tissues of cattle and swine of 2.0 parts per million (ppm) in kidney and 0.5 ppm in other tissues, and 0.125 ppm in milk.

[59 FR 41977, Aug. 16, 1994]

§ 556.220 3,5-Dinitrobenzamide.

No residues of 3,5-dinitrobenzamide may be found in the uncooked edible tissues of chickens as determined by the following method of analysis:

I. *Method of analysis—3,5-dinitrobenzamide.* A method for 3,5-dinitrobenzamide (3,5-DNBA) in chicken tissues is described with a cleanup step that removes most of the interfering materials, thus allowing uncompensated measurements to be read. The 3,5-DNBA is extracted from the sample with acetone and chloroform and prepared for chromatography by removing the aqueous phase in a separatory funnel and the solvents in a flash evaporator. The extract residue is chromatographed on alumina to remove several lipid components and residues of other drugs. The benzamide eluate is passed through a column of Dowex-50 resin, or equivalent, to remove arylamines; for example, 3-amino-5-nitrobenzamide. The 3,5-DNBA fraction is reduced, after removal of alcohol, with $TiCl_3$ in basic solution to an arylamine, presumably 3,5-diaminobenzamide. The reduced fraction is placed on another Dowex-50 column, most of the interfering substances are removed with washings of alcohol and water, and the arylamine residue is eluted with 4*N* HCl. Colorimetric measurement is made in a 100-millimeter cell at 530 millimicrons after reacting the residue with Bratton-Marshall reagents.

II. *Reagents.* A. Acetone.

B. Acetyl-(*p*- nitrophenyl)-sulfanilamide (APNPS) standard—melting point range 264° C.–267° C. Weigh and transfer 10 milligrams of APNPS to a 100-milliliter flask, dissolve and dilute to volume with acetone.

C. Alumina—activated F–20, 80–200 mesh, Aluminum Co. of America, or equivalent substance.

D. Ammonium sulfamate.

E. Ammonium sulfamate solution 1.25 grams of ammonium sulfamate per 100 milliliters of water. Refrigerate when not in use. Prepare fresh weekly.

F. Cation-exchange resin—Dowex 50W–X8, 200–400 mesh, Baker Analyzed Reagent, or equivalent, prepared as follows:

1. Place 500 grams of resin into a 3-liter beaker.
2. Add 2,000 milligrams of 6*N* HCl.
3. Heat and stir while on a bath at 80° C. for 6 hours. Discontinue heating and continue stirring overnight.
4. Filter the resin on a Buchner funnel (24 cm.) fitted with Whatman No. 1 paper.
5. Wash the resin bed with four 500-milliliter portions of 6*N* HCl.
6. Wash the resin bed with 500-milliliter portions of deionized water until the effluent has a pH of 5 or higher.
7. Wash the resin bed with three 400-milliliter portions of specially denatured alcohol 3A. Drain thoroughly.
8. Make a slurry of resin in 1,250 milliliters of specially denatured alcohol 3A.

G. Chloroform.

H. Coupling reagent—0.25 gram of *N*-1-naphthyl-ethylenediamine dihydrochloride per 100 milliliters of water. Refrigerate when not in use. Prepare fresh weekly.

I. 3,5-Dinitrobenzamide (3,5-DNBA standard). Add to boiling specially denatured alcohol 3A until a saturated solution is obtained and treat with activated carbon, filtered and

crystallize by cooling to room temperature. The 3,5-DNBA therefrom is treated a second time with activated carbon and then recrystallized three more times from specially denatured alcohol 3A. The third crystallization is washed with diethyl ether and dried in a vacuum desiccator, melting point range 185° C.–186° C.

J. Ethyl alcohol—absolute, A.C.S.

K. Eluting reagent A. The formula and volume required in procedure step V–D is dependent on the adsorptive strength of the Al_2O_3. For each lot Al_2O_3, make the following test:

1. Prepare a column (see procedure step V–D for determining formula and volume to eluting reagent A).

2. Transfer 1 milliliter of APNPS standard (100 micrograms per milliliter) in 75 milliliters of chloroform to the column.

3. Wash the column with 100 milliliters of chloroform and discard the eluate.

4. Pass through 100 milliliters of solution consisting of specially denatured alcohol 3A and ethyl alcohol 1:1 (volume to volume). Collect one 50-milliliter and five 10-milliliter portions; these make up the first, second, third, fourth, fifth, and sixth portions of eluate.

5. Place in beakers under a stream of air on a water bath (90° C.) until the solvents are evaporated.

6. Add 10 milliliters of 4*N* HCl to each, cover with watch glasses and heat (90° C.) for 30 minutes; cool to room temperature.

7. Add the Bratton-Marshall reagents.

8. All fractions show a slight color. Note the portion containing the first significant increase in pink color.

a. If the color increases in the second, third, or fourth portions of eluate, the formula in procedure step V–D is suitable and, depending on the portion, 45, 55, or 65 milliliters, respectively, should be used in procedure step V–D4. Thereby, the APNPS is retained on the column and the benzamides are eluted.

b. If the color increases in the first portion, the eluting strength of the reagent is too strong. Return the test, substituting 1:4 (volume to volume) in procedure step V–D4. If 1:4 (volume to volume) is too strong, rerun with ethyl alcohol in procedure step V–D. If none of these are suitable, another lot of Al_2O_3 should be used.

c. If the color increases in the fifth or sixth portion, the eluting strength of the reagent is too weak. Rerun the test, substituting in procedure step V–D4, respectively, 4:1 (volume to volume), specially denatured alcohol 3A: methyl alcohol, 4:1 (volume to volume), until a suitable formula is found. If none of these are suitable, another lot of Al_2O_3 should be used.

L. Hydrochloric acid, 4*N*. Add two volumes of water to one volume of HCl.

M. Diatomaceous earth—Hyflo Super Cel, Johns-Manville Co., or equivalent substance.

N. *N*-1-Naphthylethylenediamine dihydrochloride.

O. Sodium hydroxide solution, 10*N*. Dissolve 100 grams of sodium hydroxide in water and dilute to 25 milliliters.

P. Sodium nitrite solution—0.25 grams of sodium nitrite per 100 milliliters of water. Refrigerate when not in use. Prepare fresh weekly.

Q. Specially denatured alcohol, formula 3A–100 parts of 190-proof ethyl alcohol plus 5 parts of commercial methyl alcohol.

R. Titanium(ous) chloride–20 percent solution.

III. *Special apparatus.* A. Absorption cells—Beckman No. 75195 matched set of two cylindrical silica cells with 100 millimeter optical length, or equivalent cells.

B. Autotransformer—type 500B, or equivalent. To regulate speed of mixer.

C. Centrifuge.

D. Centrifuge tubes—50-milliliter size with glass stopper.

E. Chromatography tubes—Corning No. 38460, 20 millimeters A 400 millimeters and having a tapered 29/42 joint with coarse, fritted disc, or equivalent tubes.

F. Evaporator—vacuum, rotary, thin film.

G. Ion-exchange column—as described by Thiegs et al. in ''Determination of 3-amino-5-nitro-*o*- toluamide (ANOT) in chicken tissues'' published in ''Journal of Agricultural and Food Chemistry,'' volume 9, pages 201–204 (1961).

H. Glycerol manostat. For regulating pressure on columns: To Al_2O_3 columns, 15-inch head pressure; to ion-exchange columns, 30-inch head pressure.

I. Motor speed control. For regulating speed on 1-quart blender.

J. Volumetric flasks—50 milliliter size, actinic ware.

K. Mixer—Vortex Jr. Model K–500–1, Scientific Industries, Inc., or equivalent mixer.

L. One-quart blender.

M. Water bath (45° C.–50° C.).

N. Water bath (90° C.).

IV. *Standard curve.* A. 1. Weigh 100 milligrams of 3,5-DNBA and transfer to a 1-liter volumetric flask with acetone.

2. Dissolve and dilute with acetone to volume.

3. Dilute 1 milliliter to 100 milliliters.

4. Add 5.0 milliliters of water to each of six centrifuge tubes.

5. Add standard to each of the tubes to contain one of the following amounts: 0.0, 1.0, 2.0, 3.0, 5.0, and 10.0 micrograms of 3,5-DNBA.

B. Prepare each tube for colorimetric measurement as follows:

1. Place the tube in a hot water bath (90° C.) until 5.0 milliliters remain. Cool to room temperature.

2. While mixing on Vortex mixer, or equivalent, regulated with an autotransformer,

add 2 drops of $TiCl_3$ and 4 drops of 10*N* NaOH. Continue mixing until chalky-white in appearance.

3. Add 2 milliliters of HCl, mix, and allow to stand for 5 minutes.

4. Transfer to 50-milliliter volumetric flask and dilute with 4*N* HCl to 40–45 milliliters.

5. Cool to 0° C.–5° C. by placing in a freezer or ice bath.

6. Perform the Bratton-Marshall reaction in subdued light as follows:

a. Add 1 milliliter of sodium nitrite reagent, mix, and allow to stand for 1 minute.

b. Add 1 milliliter of ammonium sufamate reagent, mix, and allow to stand for 1 minute.

c. Add 1 milliliter of coupling reagent, mix, and allow to stand for 10 minutes.

d. Dilute to volume with 4*N* HCl.

C. Perform colorimetric measurement at 530 millimicrons as follows:

1. Fill two matched 100-millimeter cells with 4*N* HCl and place into spectrophotometer.

2. Adjust dark current.

3. Adjust to zero absorbance.

4. Replace acid in cell of sample side of compartment with standard to be measured.

5. The standard curve should be run five different times. Plot equivalent concentration in tissue versus mean absorbance at each concentration. If computer is available, a better procedure is to calculate the equation of the standard curve by means of least squares.

V. *Procedure.* A. Extraction. 1. Mince 350 grams of tissue in a 1-quart blending jar for 3 minutes. Use samples obtained from either freshly killed or quickly frozen birds. The latter should be analyzed as soon as thawed. For fibrous meats (for example, muscle, skin) put through a meat grinder before mincing.

2. Weight 100±0.5 grams of each replicate sample in a 150-milliliter beaker. Analyze each sample in triplicate and average the results. Reproducibility of ±10 percent between such analyses has been obtained.

3. Transfer the sample to a 1-quart blender jar. For kidney and liver tissues, make a slurry with acetone in the weighing beaker. Transfer with several rinses of acetone.

4. Blend the sample for 5 minutes with 250 milliliters of acetone and a 100-milliliter beakerful of diatomaceous earth.

5. Filter through a Buchner funnel containing a wetted Whatman No. 5 filter paper (12.5 cm.) into a 1-liter suction flask.

6. Rinse the blender jar into the funnel with three 25-milliliter portions of acetone.

7. Transfer the pulp and paper from the funnel to the aforementioned blender jar.

8. Add 250 milliliters of chloroform.

9. Blend for 3 minutes.

10. Filter through the aforementioned apparatus of procedure step V–A5. For rapid filtration of skin and blood samples, prepare funnel by adding diatomaceous earth and tamping evenly over paper to a thickness of 3 to 5 millimeters.

11. Rinse the blender jar into the funnel with three 25-milliliter rinses of chloroform.

B. Phasic separation. 1. Pour the combined filtrates into a 1-liter separatory funnel.

2. Rinse the suction flask twice with 25 milliliters of chloroform.

3. Mix the funnel contents by gently rocking and swirling for 30 seconds.

4. Let stand 10 minutes to allow phases to separate.

a. The upper (aqueous) phase (30 to 50 milliliters) is not always emulsion-free. Losses from emulsions have not been significant.

b. If an upper (aqueous) phase does not appear, add an additional 100 milliliters of chloroform and 10 milliliters of water and repeat procedure step V–B3.

5. Withdraw the lower phase into a 1-liter round-bottom flask, and discard upper phase. Withdraw nearly all of the lower phase, let stand for 2 to 3 minutes, then withdraw the remainder.

C. Evaporation. Attach the flask on a thin-film rotary evaporator connected to a vacuum supply, and place in a water bath maintained at 45° C.–50° C. until an oily residue remains. Do not overheat the sample or allow to go to dryness.

D. Adsorption chromatography. 1. Prepare a chromatography column using a column with calibrated etchings to indicate appropriate adsorbent and solvent levels as follows:

a. Fill tube to a depth of 60 millimeters with Al_2O_3.

b. Tap walls gently with hands.

c. Add anhydrous sodium sulfate to an additional depth of 25 millimeters.

d. Wet and wash column with 50 milliliters of chloroform.

i. During chromatography, make each addition to the tube when the liquid level has reached the top of the sodium sulfate layer.

ii. Increase the percolation rates by applying a slight air pressure to the top of the column.

2. Transfer the residue from procedure step V–C to the column with four 15-milliliter rinses of chloroform. Then rinse the walls of the tube and sodium sulfate layer with three 5-milliliter portions of chloroform. Percolation rate: 15 to 25 milliliters per minute. No color from sample should be seen in sodium sulfate layer after final rinse.

3. Wash column with 100 milliliters of chloroform. Discard eluate.

4. Add 75 milliliters of eluting reagent A and collect eluate A in a 250-milliliter beaker for cation-exchange chromatography.

a. Refer to ''Eluting reagent A'' under ''Reagents'' (II–K) for determining formula and volume.

b. Percolation rate: 8 to 12 milliliters per minute.

E. Cation-exchange chromatography—No. 1. 1. Prepare an ion-exchange column as follows:

a. Add a uniform slurry of resin to the column to obtain a 4 to 5 centimeter bed depth after settling.

i. Obtain a uniform slurry using a magnetic stirrer. To add the required amount of resin, calibrate the slurry and transfer it with a 10-milliliter pipette to deliver a reproducible volume.

ii. Increase the flow rate to 2 to 4 milliliters per minute by applying air pressure to the column. A glycerol manostat adjusted to 30 inches and attached between an air supply and column provides adequate pressure.

b. Wash the resin with 10 milliliters of eluting reagent A. Discard eluate.

2. Pass eluate A from procedure step V–D4 through the column. Collect in a 250-milliliter beaker.

3. Pass 50 milliliters of specially denatured alcohol 3A through the column. Combine with the eluate of procedure step V–E2.

F. Reduction. 1. Place the eluate A fraction from procedure step V–E3 on a hot water bath (90° C.) and evaporate with a stream of air until 5 to 10 milliliters remain. Do not overheat the sample or allow the sample to go to dryness.

2. Transfer to centrifuge tube and rinse beaker three times with 3 milliliters of specially denatured alcohol 3A.

3. Evaporate on a hot water bath (90° C.) under a stream of air until alcohol has evaporated. Do not overheat the sample or allow the sample to go to dryness.

4. Remove the tube from the water bath and immediately add 5.0 milliliters of water.

5. While mixing, add 2 drops of titanium chloride and 4 drops of 10*N* sodium hydroxide. Continue mixing until greyish color disappears.

a. Mix on Vortex Jr. mixer, or equivalent, regulated with autotransformer.

b. Precipitate of insoluble tissue substances and white titanium salts is present after reduction is complete.

6. Dilute to 50 milliliters with specially denatured alcohol 3A and mix.

7. Centrifuge for 5 minutes at 2,000 r.p.m.

G. Cation-exchange chromatography—No. 2. 1. Prepare resin column by procedure step V–E.

2. Pass the centrifugate of procedure step V–F7 through column. Use three rinses of specially denatured alcohol 3A, each 5 milliliters, to aid in transferring of sample.

3. Pass 50 milliliters of specially denatured alcohol 3A through the column.

4. Pass 50 milliliters of deionized water through the column.

5. Elute arylamine residue from the resin with 40 to 43 milliliters of 4*N* HCl into a 50-milliliter volumetric flask (actinic ware) for 3,5-DNBA analysis. Avoid direct sunlight. The arylamine has been found to be photosensitive.

H. Color development and measurement. 1. Cool to 0° C.–5° C. by placing in a freezer or ice bath.

2. Perform the Bratton-Marshall reaction in subdued light as follows:

a. Add 1 milliliter of sodium nitrite reagent, mix, and allow to stand for 1 minute.

b. Add 1 milliliter of ammonium sulfamate reagent, mix, and allow to stand for 1 minute.

c. Add 1 milliliter of coupling reagent, mix, and allow to stand for 10 minutes.

d. Dilute to volume with 4*N* HCl.

3. Perform colorimetric measurement at 530 millimicrons as follows:

a. Fill two matched 100-millimeter cells with 4*N* HCl and place into instrument.

b. Adjust dark current.

c. Adjust to zero absorbance.

d. Replace acid in cell of sample side of compartment with sample to be measured.

e. Record absorbance observed.

I. Calculations. Determine parts per billion (observed) from the standard curve.

§ 556.230 Erythromycin.

Tolerances for residues of erythromycin in food are established as follows:

(a) 0.1 part per million in uncooked edible tissues of beef cattle and swine.

(b) Zero in milk.

(c) 0.025 part per million in uncooked eggs.

(d) 0.125 part per million (negligible residue) in uncooked edible tissues of chickens and turkeys.

[40 FR 13942, Mar. 27, 1975, as amended at 58 FR 43795, Aug. 18, 1993]

§ 556.240 Estradiol and related esters.

No residues of estradiol, resulting from the use of estradiol or any of the related esters, are permitted in excess of the following increments above the concentrations of estradiol naturally present in untreated animals:

(a) In uncooked edible tissues of heifers, steers, and calves:

(1) 120 parts per trillion for muscle.

(2) 480 parts per trillion for fat.

(3) 360 parts per trillion for kidney.

(4) 240 parts per trillion for liver.

(b) In uncooked edible tissues of lambs:

(1) 120 parts per trillion for muscle.

(2) 600 parts per trillion for fat, kidney, and liver.

[49 FR 13873, Apr. 9, 1984, as amended at 56 FR 67175, Dec. 30, 1991]

§ 556.260 Ethopabate.

Tolerance for residues of ethopabate converted to metaphenetidine are established in the edible tissues of chickens as follows:

(a) 1.5 parts per million in uncooked liver and kidney.

(b) 0.5 part per million in uncooked muscle.

§ 556.270 Ethylenediamine.

A tolerance of zero is established for residues of ethylenediamine in milk.

§ 556.275 Fenbendazole.

(a) *Cattle and goats.* A tolerance[1] of 0.8 part per million is established for parent fenbendazole (the marker residue) in the liver of cattle and goats.

(b) *Swine.* A tolerance[1] for marker residues of fenbendazole in swine is not needed.

[59 FR 26943, May 25, 1994]

§ 556.277 Fenprostalene.

A tolerance for marker residue of fenprostalene in cattle is not needed. The safe concentrations for the total residues of fenprostalene in the uncooked edible tissues of cattle are 10 parts per billion in muscle, 20 parts per billion in liver, 30 parts per billion in kidney, 40 parts per billion in fat, and 100 parts per billion in the injection site. As used in this section ''tolerance'' refers to a concentration of a marker residue in the target tissue selected to monitor for total residues of the drug in the target animal, and ''safe concentrations'' refer to the concentrations of total residues considered safe in edible tissues.

[49 FR 26716, June 29, 1984]

§ 556.290 Furazolidone.

A tolerance of zero is established for residues of furazolidone in the uncooked edible tissues of swine.

§ 556.300 Gentamicin sulfate.

(a) A tolerance of 0.1 part per million is established for negligible residues of gentamicin sulfate in the uncooked edible tissues of turkeys.

(b) Tolerances are established for total residues of gentamicin in edible tissues of swine as follows: 0.1 part per million in muscle, 0.3 part per million in liver, and 0.4 part per million in fat and kidney. A microbiological determinative procedure and an HPLC confirmatory procedure for gentamicin have been developed to assay gentamicin in kidney at 0.4 ppm. Since residues of gentamicin as the parent compound and total residues are equal, the marker (parent drug) residue concentration of 0.4 ppm in kidney corresponds to 0.4 ppm of total residue.

[48 FR 791, Jan. 7, 1983]

§ 556.308 Halofuginone hydrobromide.

The marker residue selected to monitor for total residues of halofuginone hydrobromide in broilers and turkeys is parent halofuginone hydrobromide and the target tissue selected is liver. A tolerance is established in broilers of 0.16 part per million and in turkeys of 0.13 part per million for parent halofuginone hydrobromide in liver. These marker residue concentrations in liver correspond to total residue concentrations of 0.3 part per million in liver. The safe concentrations for total residues of halofuginone hydrobromide in the uncooked edible tissues of broilers and turkeys are 0.1 part per million in muscle, 0.3 part per million in liver, and 0.2 part per million in skin with adhering fat. As used in this section, ''tolerance'' refers to a concentration of a marker residue in the target tissue selected to monitor for total residues of the drug in the target animal, and ''safe concentrations'' refers to the concentrations of total residues considered safe in edible tissues.

[54 FR 28052, July 5, 1989, as amended at 56 FR 8711, Mar. 1, 1991; 57 FR 21209, May 19, 1992]

§ 556.310 Haloxon.

A tolerance of 0.1 part per million is established for negligible residues of haloxon (3-chloro-7-hydroxy-4-methyl-

[1] As used in this section: ''tolerance'' refers to a concentration of a marker residue in the target tissue selected to monitor for total residues of the drug in the target animal.

coumarin bis(2-chloroethyl) phosphate) in the edible tissues of cattle.

[40 FR 13942, Mar. 27, 1975, as amended at 45 FR 10333, Feb. 15, 1980]

§ 556.320 Hydrocortisone.

A tolerance is established for negligible residues of hydrocortisone (as hydrocortisone sodium succinate or hydrocortisone acetate) in milk at 10 parts per billion.

§ 556.330 Hygromycin B.

A tolerance of zero is established for residues of hygromycin B in or on eggs and the uncooked edible tissues of swine and poultry.

§ 556.344 Ivermectin.

The marker residue tolerance and safe concentrations for total residues in edible tissues of target animals are as follows:

(a) *Cattle.* The marker residue used to monitor the total residues of ivermectin in cattle is 22,23-dihydroavermectin B_1a. The target tissue selected is liver. A tolerance is established for 22,23-dihydro-avermectin B_1a in cattle of 100 parts per billion in liver. A marker residue concentration of 100 parts per billion in liver corresponds to a concentration for total residues of ivermectin of 240 parts per billion in liver. The safe concentrations for total residues of ivermectin in uncooked, edible tissues of cattle is 120 parts per billion in muscle, 240 parts per billion in liver, 360 parts per billion in kidney, and 480 parts per billion in fat.

(b) *Swine.* The marker residue used to monitor the total residues of ivermectin in swine 22,23-dihydroavermectin B_1a. The target tissue selected is liver. A tolerance is established for 22,23-dihydro-avermectin B_1a in swine of 20 parts per billion in liver. A marker residue concentration of 20 parts per billion in liver corresponds to a concentration for total residues of ivermectin of 75 parts per billion in liver. The safe concentrations for total residues of ivermectin in uncooked edible tissues of swine are 25 parts per billion in muscle, 75 parts per billion in liver, 100 parts per billion in kidney, and 100 parts per billion in fat.

(c) *Sheep.* The marker residue used to monitor the total residues of ivermectin in sheep is 22,23-dihydroavermectin B_1a (H_2B_1a). The target tissues selected is liver. A tolerance is established for H_2B_1a in sheep of 30 parts per billion in liver. A marker residue concentration of 30 parts per billion in liver corresponds to a concentration for total residues of ivermectin of 125 parts per billion in liver. The safe concentrations for total residues of ivermectin in uncooked edible tissues of sheep are 25 parts per billion in muscle and 125 parts per billion in liver, kidney, and fat.

(d) *Reindeer.* The marker residue used to monitor the total residues of ivermectin in reindeer is 22,23-dihydroavermectin B_1a. The target tissue selected is liver. A tolerance is established for 22,23-dihydro-avermectin B_1a in reindeer of 15 parts per billion in liver. A marker residue concentration of 15 parts per billion in liver corresponds to a concentration for total residues of ivermectin of 50 parts per billion in liver. The safe concentrations for total residues of ivermectin in uncooked, edible tissues of reindeer are 25 parts per billion in muscle, 50 parts per billion in liver, 75 parts per billion in kidney, and 100 parts per billion in fat.

[51 FR 27021, July 29, 1986, as amended at 53 FR 27958, July 26, 1988; 59 FR 50830, Oct. 6, 1994]

§ 556.347 Lasalocid.

As used in this section ''tolerance'' refers to a concentration of a marker residue in the target tissue selected to monitor for total residues of the drug in the target animal, and ''safe concentrations'' refers to the concentrations of total residues considered safe in edible tissues.

(a) *Chickens.* The marker residue selected to monitor for total residues of lasalocid in chickens is parent lasalocid. The target tissue is skin with adhering fat. A tolerance for the marker is established in chickens of 0.3 part per million for parent lasalocid in skin with adhering fat. A marker residue concentration of 0.3 part per million in skin with adhering fat corresponds to a concentration for total residues of lasalocid of 7.2 parts per

million in liver. The safe concentrations for total residues of lasalocid in the uncooked edible tissues of chickens are 1.2 parts per million in muscle, 2.4 parts per million in skin with adhering fat, and 7.2 parts per million in liver.

(b) *Cattle.* The marker residue selected to monitor for total residues of lasalocid sodium in cattle is parent lasalocid and the target tissue selected is the liver. A tolerance is established in cattle of 0.7 part per million for parent lasalocid in liver. A marker residue concentration of 0.7 part per million in liver corresponds to a concentration for total residues of lasalocid of 4.8 parts per million in liver. The safe concentrations for total residues of lasalocid in the uncooked edible tissues of cattle are 1.2 parts per million in muscle, 4.8 parts per million in liver and in fat, and 3.6 parts per million in kidney.

(c) *Sheep.* A tolerance for marker residues of lasalocid sodium in sheep is not needed. The safe concentrations for total residues of lasalocid in the uncooked edible tissues of sheep are 1.2 parts per million in muscle and 6 parts per million in liver, fat, and kidney.

[49 FR 27316, July 3, 1984, as amended at 49 FR 29057, July 18, 1984]

§ 556.350 Levamisole hydrochloride.

A tolerance of 0.1 part per million is established for negligible residues of levamisole hydrochloride in the edible tissues of cattle, sheep, and swine.

§ 556.360 Lincomycin.

(a) *Swine.* A tolerance of 0.1 part per million is established for negligible residues in the edible tissues.

(b) *Chickens.* A tolerance for residues of lincomycin in chickens is not required.

[55 FR 3209, Jan. 31, 1990]

§ 556.375 Maduramicin ammonium.

A tolerance is established for residues of maduramicin ammonium in chickens as follows:

(a) A tolerance for maduramicin ammonium (marker residue) in chickens is 0.38 parts per million in fat (target tissue). A tolerance refers to the concentration of marker residues in the target tissue used to monitor for total drug residues in the target animals.

(b) The safe concentrations for total maduramicin ammonium residues in uncooked edible chicken tissues are: 0.24 parts per million in muscle; 0.72 parts per million in liver; 0.48 parts per million in skin; and 0.48 parts per million in fat. A safe concentration refers to the total residue concentration considered safe in edible tissues.

[54 FR 5229, Feb. 2, 1989]

§ 556.380 Melengestrol acetate.

A tolerance of 25 parts per billion is established for residues of the parent compound, melengestrol acetate, in fat of cattle.

[59 FR 41241, Aug. 11, 1994]

§ 556.390 Methylparaben.

A tolerance of zero is established for residues of methylparaben in milk from dairy animals.

§ 556.400 Methylprednisolone.

A tolerance is established for negligible residues of methylprednisolone in milk at 10 parts per billion.

§ 556.410 Metoserpate hydrochloride.

A tolerance of 0.02 part per million is established for negligible residues of metoserpate hydrochloride (methyl-*o*-methyl-18-epireserpate hydrochloride) in uncooked edible tissues of chickens.

§ 556.420 Monensin.

(a) *Cattle and goats.* A tolerance of 0.05 part per million is established for negligible residues of monensin in the edible tissues of cattle and goats.

(b) *Chickens, turkeys, and quail.* A tolerance for marker residues of monensin in chickens, turkeys, and quail is not needed. The safe concentrations for total residues of monensin in chickens, turkeys, and quail are 1.5 parts per million in muscle, 3.0 parts per million in skin with adhering fat, and 4.5 parts per million in liver. *Tolerance* in this paragraph refers to the concentration of a marker residue in the target tissue selected to monitor for total residues of the drug in the target animals. *Safe concentrations* refers to the

concentration of total residues considered safe in edible tissues.

[50 FR 32394, Aug. 12, 1985, as amended at 52 FR 15718, Apr. 30, 1987; 53 FR 40060, Oct. 13, 1988; 54 FR 32633, Aug. 9, 1989]

§ 556.425 Morantel tartrate.

A tolerance of 0.7 part per million is established for *N*-methyl-1,3-propanediamine (MAPA, marker residue) in the liver (target tissue) of cattle and goats. A tolerance for residues of morantel tartrate in milk is not required.

[59 FR 17922, Apr. 15, 1994]

§ 556.428 Narasin.

A tolerance for narasin residues in chickens is not needed. The safe concentrations for total narasin residues in uncooked edible chicken tissues are: 0.6 part per million in muscle; 1.8 parts per million in liver; 1.2 parts per million in skin with adhering fat and fat. A tolerance refers to the concentration of marker residues in the target tissue used to monitor for total drug residues in the target animals. A safe concentration refers to the total residue concentration considered safe in edible tissues.

[51 FR 29097, Aug. 14, 1986]

§ 556.430 Neomycin.

Tolerances are established for residues of neomycin in food as follows: 0.25 part per million (negligible residue) in edible tissues of calves; and 0.15 part per million (negligible residue) in milk.

§ 556.440 Nequinate.

A tolerance of 0.1 part per million is established for negligible residues of nequinate in the uncooked edible tissues of chickens.

§ 556.445 Nicarbazin.

A tolerance of 4 parts per million is established for residues of nicarbazin in uncooked chicken muscle, liver, skin, and kidney.

[42 FR 56729, Oct. 28, 1977]

§ 556.460 Novobiocin.

Tolerances for residues of novobiocin are established at 0.1 part per million in milk from dairy animals and 1 part per million in the uncooked edible tissues of cattle, chickens, turkeys, and ducks.

[47 FR 18590, Apr. 30, 1982]

§ 556.470 Nystatin.

A tolerance of zero is established for residues of nystatin in or on eggs and the uncooked edible tissues of swine and poultry.

§ 556.480 Oleandomycin.

Tolerances are established for negligible residues of oleandomycin in uncooked edible tissues of chickens, turkeys, and swine at 0.15 part per million.

§ 556.490 Ormetoprim.

A tolerance of 0.1 part per million is established for negligible residues of ormetoprim in the edible tissues of chickens, turkeys, ducks, salmonids, and catfish.

[51 FR 18884, May 23, 1986]

§ 556.495 Oxfendazole.

Cattle: A tolerance is established for total oxfendazole residues in edible cattle tissues based on a marker residue concentration of 0.8 part per million (ppm) fenbendazole in the target liver tissue. A fenbendazole concentration of 0.8 ppm in liver corresponds to a total safe concentration of oxfendazole residues of 1.7 ppm in liver. The safe concentrations of total oxfendazole residues in other uncooked edible cattle tissues are: muscle, 0.84 ppm; kidney, 2.5 ppm; and fat, 3.3 ppm. A tolerance refers to the concentration of marker residue in the target tissue selected to monitor for total drug residue in the target animal. A safe concentration is the total residue considered safe in edible tissue.

[55 FR 46943, Nov. 8, 1990]

§ 556.500 Oxytetracycline.

Tolerances are established for residues of oxytetracycline in food as follows:

(a) In edible tissues of chickens and turkeys:

(1) 3 parts per million in uncooked kidney.

(2) 1 part per million in uncooked muscle, liver, fat, and skin.

(b) 0.1 part per million in uncooked edible tissues of swine.

(c) 0.1 part per million in uncooked edible tissues of cattle, beef calves, nonlactating dairy cattle and dairy calves.

(d) A tolerance of 0.1 part per million is established for negligible residues of oxytetracycline in uncooked edible tissues of salmonids, catfish, and lobsters.

(e) 0.1 part per million in uncooked edible tissues of sheep.

[40 FR 13942, Mar. 27, 1975, as amended at 52 FR 24293, June 30, 1987; 58 FR 42855, Aug. 12, 1993]

§ 556.510 Penicillin.

Tolerances are established for residues of penicillin and the salts of penicillin in food as follows:

(a) 0.05 part per million (negligible residue) in the uncooked edible tissues of cattle.

(b) Zero in the uncooked edible tissues of chickens, pheasants, quail, swine, and sheep; in eggs; and in milk or in any processed food in which such milk has been used.

(c) 0.01 part per million in the uncooked edible tissues of turkeys.

[40 FR 13942, Mar. 27, 1975, as amended at 43 FR 32749, July 28, 1978]

§ 556.515 Pirlimycin.

A tolerance is established for residues of parent pirlimycin (marker substance) in cattle liver (target tissue) of 0.5 part per million and in milk of 0.4 part per million.

[58 FR 58486, Nov. 2, 1993]

§ 556.520 Prednisolone.

A tolerance of zero is established for residues of prednisolone in milk from dairy animals.

§ 556.530 Prednisone.

A tolerance of zero is established for residues of prednisone in milk from dairy animals.

§ 556.540 Progesterone.

No residues of progesterone are permitted in excess of the following increments above the concentrations of progesterone naturally present in untreated animals:

(a) In uncooked edible tissues of steers and calves:

(1) 3 parts per billion for muscle.

(2) 12 parts per billion for fat.

(3) 9 parts per billion for kidney.

(4) 6 parts per billion for liver.

(b) In uncooked edible tissues of lambs:

(1) 3 parts per billion for muscle.

(2) 15 parts per billion for fat, kidney, and liver.

[49 FR 13873, Apr. 9, 1984]

§ 556.550 Propylparaben.

A tolerance of zero is established for residues of propylparaben in milk from dairy animals.

§ 556.560 Pyrantel tartrate.

Tolerances are established for residues of pyrantel tartrate in edible tissues of swine as follows:

(a) 10 parts per million in liver and kidney.

(b) 1 part per million in muscle.

§ 556.580 Robenidine hydrochloride.

Tolerances are established for residues of robenidine hydrochloride in edible tissues of chickens as follows:

(a) 0.2 part per million in skin and fat.

(b) 0.1 part per million (negligible residue) in edible tissues other than skin and fat.

§ 556.590 Salicylic acid.

A tolerance of zero is established for residues of salicylic acid in milk from dairy animals.

§ 556.594 Sarafloxacin.

A tolerance for residues of sarafloxacin in edible turkey and broiler chickens tissues is not required.

[60 FR 50098, Sept. 28, 1995]

§ 556.600 Spectinomycin.

A tolerance of 0.1 part per million is established for negligible residues of spectinomycin in the uncooked edible tissues of chickens.

§ 556.610 Streptomycin.

Tolerances are established for residues of streptomycin in uncooked,

edible tissues of chickens, swine, and calves of 2.0 parts per million (ppm) in kidney and 0.5 ppm in other tissues.

[58 FR 47211, Sept. 8, 1993]

§ 556.620 Sulfabromomethazine sodium.

Tolerances for residues of sulfabromomethazine sodium in food are established as follows:

(a) In the uncooked edible tissues of cattle at 0.1 part per million (negligible residue).

(b) In milk at 0.01 part per million (negligible residue).

[47 FR 30244, July 13, 1982]

§ 556.625 Sodium sulfachloropyrazine monohydrate.

A tolerance of zero is established for residues of sodium sulfachloropyrazine monohydrate in the uncooked edible tissues of chickens.

§ 556.630 Sulfachlorpyridazine.

A tolerance of 0.1 part per million is established for negligible residues of sulfachlorpyridazine in uncooked edible tissues of calves and swine.

§ 556.640 Sulfadimethoxine.

Tolerances are established for residues of sulfadimethoxine in edible products of animals as follows:

(a) In the uncooked edible tissues of chickens, turkeys, cattle, ducks, salmonids, and catfish at 0.1 part per million (negligible residue).

(b) In milk at 0.01 part per million (negligible residue).

[40 FR 13942, Mar. 27, 1975, as amended at 49 FR 46371, Nov. 26, 1984; 51 FR 18884, May 23, 1986]

§ 556.650 Sulfaethoxypyridazine.

Tolerances for residues of sulfaethoxypyridazine in food are established as follows:

(a) Zero in the uncooked edible tissues of swine and in milk.

(b) 0.1 part per million (negligible residue) in uncooked edible tissues of cattle.

§ 556.660 Sulfamerazine.

A tolerance of zero is established for residues of sulfamerazine (N^1-[4-methyl-2-pyrimidinyl]sulfanilamide) in the uncooked edible tissues of trout.

§ 556.670 Sulfamethazine.

A tolerance of 0.1 part per million is established for negligible residues of sulfamethazine in the uncooked edible tissues of chickens, turkeys, cattle, and swine.

[47 FR 25323, June 11, 1982]

§ 556.680 Sulfanitran.

A tolerance of zero is established for residues of sulfanitran (acetyl(*p*- nitrophenyl) sulfanilamide) and its metabolites in the uncooked edible tissues of chickens.

§ 556.690 Sulfathiazole.

A tolerance of 0.1 part per million is established for negligible residues of sulfathiazole in the uncooked edible tissues of swine.

§ 556.700 Sulfomyxin.

A tolerance of zero is established for residues of sulfomyxin (N-sulfomethylpolymyxin B sodium salt) in uncooked edible tissues from chickens and turkeys.

§ 556.710 Testosterone propionate.

No residues of testosterone, resulting from the use of testosterone propionate, are permitted in excess of the following increments above the concentrations of testosterone naturally present in untreated animals:

(a) In uncooked edible tissues of heifers:

(1) 0.64 part per billion in muscle.

(2) 2.6 parts per billion in fat.

(3) 1.9 parts per billion in kidney.

(4) 1.3 parts per billion in liver.

(b) [Reserved]

[52 FR 27683, July 23, 1987]

§ 556.720 Tetracycline.

A tolerance of 0.25 part per million is established for negligible residues of tetracycline in uncooked edible tissues of calves, swine, sheep, chickens, and turkeys.

§ 556.730 Thiabendazole.

Tolerances are established at 0.1 part per million for negligible residues of thiabendazole in uncooked edible

tissues of cattle, goats, sheep, pheasants, and swine, and at 0.05 part per million for negligible residues in milk.

[40 FR 13942, Mar. 27, 1975, as amended at 49 FR 29958, July 25, 1984]

§ 556.735 Tilmicosin.

A tolerance of 1.2 parts per million is established for parent tilmicosin (marker residue) in liver (target tissue) of cattle.

[57 FR 12712, Apr. 13, 1992]

§ 556.738 Tiamulin.

The marker residue selected to monitor for total residues of tiamulin in swine is 8-*alpha*-hydroxymutilin and the target tissue selected is liver. A tolerance is established in swine at 0.4 part per million for 8-*alpha*-hydroxymutilin in liver. A marker residue concentration of 0.4 part per million in liver corresponds to a concentration for total residues of tiamulin of 10.8 parts per million in liver. The safe concentrations for total residues of tiamulin in the uncooked edible tissues of swine are 3.6 parts per million in muscle, 10.8 parts per million in liver, and 14.4 parts per million in kidney and fat.

[48 FR 41385, Sept. 15, 1983]

§ 556.739 Trenbolone.

A tolerance for total trenbolone residues in uncooked edible tissues of cattle is not needed. The safe concentration for total trenbolone residues in uncooked edible tissues of cattle is 50 parts per billion (ppb) in muscle, 100 ppb in liver, 150 ppb in kidney, and 200 ppb in fat. A tolerance refers to the concentration of marker residues in the target tissue used to monitor for total drug residues in the target animals. A safe concentration refers to the total residue concentration considered safe in edible tissues.

[52 FR 24995, July 2, 1987, as amended at 54 FR 12595, Mar. 28, 1989]

§ 556.740 Tylosin.

Tolerances are established for residues of tylosin in edible products of animals as follows:

(a) In chickens and turkeys: 0.2 part per million (negligible residue) in uncooked fat, muscle, liver, and kidney.

(b) In cattle: 0.2 part per million (negligible residue) in uncooked fat, muscle, liver, and kidney.

(c) In swine: 0.2 part per million (negligible residue) in uncooked fat, muscle, liver, and kidney.

(d) In milk: 0.05 part per million (negligible residue).

(e) In eggs: 0.2 part per million (negligible residue).

§ 556.750 Virginiamycin.

Tolerances are established for negligible residues of virginiamycin in edible tissues of swine as follows:

(a) Swine—

(1) 0.4 ppm in kidney, skin, and fat.

(2) 0.3 ppm in liver.

(3) 0.1 ppm in muscle.

(b) Broiler chickens—

(1) 0.5 ppm in kidney.

(2) 0.3 ppm in liver.

(3) 0.2 ppm in skin and fat.

(4) 0.1 ppm in muscle.

(c) *Cattle.* A tolerance for residues of virginiamycin in cattle is not required.

[46 FR 18966, Mar. 27, 1981, as amended at 59 FR 38902, Aug. 1, 1994]

§ 556.760 Zeranol.

(a) *Cattle.* A tolerance for total zeranol residues in uncooked edible tissues of cattle is not needed. The safe concentration for total zeranol residues in uncooked edible tissues of cattle is 150 parts per billion (ppb) in muscle, 300 ppb in liver, 450 ppb in kidney, and 600 ppb in fat. A tolerance refers to the concentration of marker residues in the target tissue used to monitor for total drug residues in the target animal. A safe concentration refers to the total residue concentration considered safe in edible tissues.

(b) *Sheep.* No residues of zeranol may be found in the uncooked edible tissues of sheep as determined by the following method of analysis:

I. METHOD OF ANALYSIS—ZERANOL

A gas chromatographic method for the determination of the drug in frozen beef tissues is described. Tissue is frozen and stored in a deep freezer until ready for examination. A weighed portion of wet tissue (with exception of fat) is homogenized and lyophilized to

dry solid. The drug is recovered from dry tissue by an extraction with methanol in a Soxhlet extractor. The methanol extract is digested in the presence of hydrochloric acid to hydrolyze conjugates should any be present. Elimination of impurities is brought about by liquid partition transfer successively to chloroform to 1*N* sodium hydroxide, to carbon tetrachloride, to 1*N* sodium hydroxide, to ethyl ether, and, finally, to a dry residue. The residue is reacted with a silane mixture to create a volatile derivative which is quantitated by peak area measurements from a flame ionization detector. The drug can be detected at a level of 20 parts per billion with negligible interference from tissues or reagents.

II. Reagents

A. Carbon tetrachloride, N.F., Fisher Scientific C–186, or equivalent.

B. Chloroform, N.F., Fisher Scientific C–296, or equivalent.

C. Chromatograph gases, flow rates adjusted to maximize sensitivity for specific chromatograph.

1. Carrier gas, conventional tank helium.

2. Flame makeup gas.

a. Oxygen, conventional tank oxygen.

b. Hydrogen, Linde high purity, or equivalent.

D. Column packing, 3 percent GE SE–52 (Applied Science Laboratories) on P.E. Celite 60–80 mesh (Johns Manville Product No. 154–0048), or equivalent.

E. Ether, anhydrous, Fisher Scientific E–138, or equivalent.

F. Hexamethyldisilazane, Dow-Corning, Peninsular, or equivalent.

G. Hydrochloric acid, analytical reagent grade.

H. Methanol, certified A.C.S., spectranalyzed, Fisher Scientific A–408, or equivalent.

I. Phosphoric acid, analytical reagent grade.

J. Pyridine, anhydrous, A.C.S. reagent grade.

K. Silating reagent mixture: Pipet 8 milliliters each of pyridine and hexamethyldisilazane and 4 milliliters of trimethylchlorosilane into a clean glass vial with a polyethylene cap and mix thoroughly. Let stand overnight and decant supernatant liquid into a vial. Cap and store at room temperature for daily use. If kept dry, the reagent is stable for more than a month. Blanks are scanned by gas chromatography on each new bottle of J, F, and N material used in the silating reagent mixture for possible peak interference in the region of zeranol derivative.

L. Sodium chloride, analytical reagent grade.

M. Sodium hydroxide, analytical reagent grade.

N. Trimethylchlorosilane, Dow-Corning, Peninsular, or equivalent.

O. Water, distilled in glass.

P. Zeranol, primary standard.

Q. Solutions.

1. 2*N* Hydrochloric acid in water.

2. 3*N* Phosphoric acid in water.

3. 2 percent w/v solium chloride in water.

4. 1*N* Sodium hydroxide in water.

III. Apparatus

A. Extraction assemblies, Soxhlet, improved, standard taper grindings, Pyrex brand glass, 1,000 milliliters capacity, Sargent Catalog S–31265D, or equivalent.

B. Flasks, freeze drying, widemouth, 1,000 milliliters capacity, 24/40 standard taper grindings, Pyrex brand glass, Sargent Catalog S–28875–20–F, or equivalent.

C. Flasks, homogenizing, 250 milliliters, Sargent Catalog S–61716, or equivalent.

D. Funnels, separatory, Squibb stopper, with Teflon stopcock plug, Pyrex brand glass, 250- and 500-milliliter capacities, Sargent Catalog S–35815–20–F or G, or equivalent.

E. Gas chromatograph, F and M Model 5750 with flame ionization detector, or equivalent.

F. Gas chromatography column: Stainless steel tubing, 6 feet by 3⁄16 inch packed with 3 percent by weight GE SE–52 (Applied Science Laboratories) deposited on P.E. Celite 60–80 mesh (product No. 154–0048), or equivalent. Condition the column by baking for 40–80 hours at 325° C. with a helium flow, but detached from the detector input. Injections of 1–2 microliters of a 50/50 mixture of hexamethyldisilazane and trimethylchlorosilane will help remove active sites in the column.

1. Prepare a TMS derivative of a 1,000-microgram zeranol standard as described in the procedure section. Inject 1-microliter quantities to determine whether the column is responding to the conditioning. After the column shows a response at the 1,000-microgram level, proceed to smaller quantities to optimize conditions.

2. The column and chromatograph must be conditioned to achieve a minimum sensitivity response so that a peak 5 millimeters in height results from an injection of 5 microliter of standard preparation containing 1 microgram of zeranol in the derivative preparation. This criterion must be met before tissue assay is attempted.

3. The column is brought to 250° C. after conditioning and held at that temperature for at least 12 hours before making a run.

G. Heating mantle, electric, Glas-Col. Sargent Catalog S–40866H, or equivalent.

H. Hot plate, with gradient rheostat heat control.

I. Meat grinder, manually operated or equivalent.

J. Steam bath.

K. Syringe, Hamilton Micro Syringe Model 701, 10-microliter capacity, or equivalent.

L. Torsion balance, 0.1 gram sensitivity, 500 grams capacity.

M. Vials, 1-dram glass with plastic tops, Owens-Illinois, Opticlear, or equivalent.

N. Virtis freeze drier, Sargent Catalog S–28881–80, or equivalent.

O. Virtis homogenizing mill, macro, Virtis No. 45, Sargent Catalog S–61700, or equivalent.

IV. Standard Solutions

A. Stock solution A: Accurately weigh 0.1000 gram of zeranol, primary standard, into a 250-milliliter beaker. Dissolve the standard in 80 milliliters of methanol and accurately dilute to 100 milliliters in a volumetric flask with methanol. By preparation, the solution contains 1,000 micrograms per milliliter.

B. Stock solution B: Dilute 10.0 milliliters of stock solution A to 100 milliliters with methanol to provide a standard containing 100 micrograms of the drug per milliliter.

C. Stock solution C: Dilute 5.0 milliliters of stock solution B to 100 milliliters with methanol to provide a standard of 5 micrograms per milliliter.

D. Stock solution D: Dilute 2.0 milliliters of stock solution B to 100 milliliters with methanol to provide a standard of 2 micrograms per milliliter. Transfer 1.0 milliliter of stock solution D to a 1-dram glass vial, evaporate to a dry residue in a vacuum desiccator at reduced pressure. The residue contains 2 micrograms of zeranol to be used as a calibration standard in operation of the gas chromatograph.

V. Procedure

A. Preparation of glassware: Glassware should be washed in detergent or chromic acid solution to remove contaminants and rinsed in water to remove traces of cleaning agent. Rinse with methanol before using.

B. Preparation of sample.

1. Collect muscle, liver, kidney, and tripe from a freshly sacrificed animal under the cleanest conditions possible.

2. Grind the fresh tissue in a meat grinder, divide into 100-gram portions, and wrap in aluminum foil. Store wrapped tissue in a deep freeze. Fat should be wrapped in foil and stored in deep freeze.

C. Extraction procedure for muscle, liver, kidney, and tripe.

1. Weight 100 grams of partially thawed tissue into a 250-milliliter homogenizing flask, add 60 milliliters of water, and attach to a Virtis ''45'' Tissue Mill, or equivalent.

2. Mix the materials at 45,000 r.p.m. for 5 minutes to obtain a thin homogenate.

3. Transfer the homogenate to a 1-liter, widemouth, freeze drying flask using 10–20 milliliters of water for a rinse.

4. Place the flask on its side in a nearly horizontal position in a slurry of dry ice and acetone. Rotate the flask on its side as the homogenate cools to set down a uniform frozen solid layer on the wall of the flask.

5. Mount the flask on a Virtis freeze drier, or equivalent, and lyophilize to dry solids. This operation usually requires 20–24 hours. *Stopping place.*

6. Transfer the solid cake to a clean sheet of paper and crumble by hand to a size convenient for transfer to an extraction thimble.

7. Transfer the solids to a single thickness 60 x 180 milliliter Soxhlet extraction thimble and compact the solids sufficiently to guarantee complete immersion during solid extraction.

8. Transfer 600 milliliters of methanol to a 1-liter pot of a Soxhlet extraction assembly and place the thimble in the extractor. Mount a large glass funnel in the neck of the extractor with the stem extending into the thimble. Rinse the 1-liter freeze drying flask with three 50-milliliter portions of fresh methanol and transfer the rinses through the funnel into the thimble. Mount the condenser in the extractor and extract the solids for 15 hours. The extractor should be heated with the electric heating mantle so that a fill-empty cycle requires 18–24 minutes.

9. Drain the methanol from the thimble. Composite the methanol from the extractor and pot in an 800-milliliter beaker.

10. Rinse the pot with 10 milliliters of methanol and add to the methanol composite. Transfer 50 milliliters of 2*N* HCl down the pot side wall, and add to methanol composite. Concentrate to 125 milliliters by boiling on a hot plate.

D. Extraction procedure for fat.

1. Cut fat into ¼-inch cubes. The lyophilization of fat is unnecessary since it is essentially water free.

2. Transfer 100 grams of the prepared fat to a 60- x 180-millimeter extraction thimble and extract with 750 milliliters of methanol for 15 hours in the Soxhlet extractor. The extractor should be heated with the electric heating mantle so that a fill-empty cycle requires 18–24 minutes.

3. Drain the methanol from the thimble. Composite the methanol from the extractor and pot into an 800-milliliter beaker.

4. Rinse the pot with 10 milliliters of methanol and add to the methanol composite. Transfer 50 milliliters of 2*N* HCl down the pot side wall, and add to methanol composite. Concentrate to 125 milliliters by boiling on a hot plate.

E. Solvent partition.

1. Transfer the methanol concentrate to a 500-milliliter separatory funnel, identified by number as 1, with 70 milliliters of chloroform rinse and mix.

2. Add 300 milliliters of water and without shaking allow liquid phases to separate.

3. Withdraw the chloroform layer into a separatory funnel, identified by number as 2, containing 100 milliliters of 2 percent aqueous sodium chloride.

4. Gently mix the contents of funnel 2 horizontally end to end 30 times and allow phases to separate. Usually about 20 minutes are required to obtain maximum chloroform separation.

5. Withdraw the chloroform layer into a beaker.

6. Extract with shaking the contents of funnels 1 and 2 successively with three more 50-milliliter portions of chloroform.

7. Composite the chloroform extracts and concentrate to 125 milliliters by evaporation on a steam bath and cool to room temperature.

8. Transfer the chloroform composite to a 250-milliliter separatory funnel, fitted with a Teflon stopcock, using 10 milliliters of chloroform as a rinse.

9. Extract the chloroform with three separate 20-milliliter portions of 1*N* sodium hydroxide solution retaining the emulsion in the sodium hydroxide phase. Agitation of sodium hydroxide with the chloroform extract for the first time is accompanied by the appearance of emulsion.

10. Perform an extraction by gently inverting the closed funnel and returning the funnel to an upright position.

11. Repeat phase mixing 30 times per extraction.

12. Allow phases to separate for 10 minutes. The time delay allows for gradual dissipation of the emulsion to improve phase separation. The zeranol transfers from the chloroform to the upper sodium hydroxide phase in this operation.

13. Composite the sodium hydroxide extracts.

14. Wash the sodium hydroxide extract with three 50-milliliter portions of chloroform using the technique as in step 9 and the same 10-minute interval for phase separation. Washing the chloroform removes the emulsion and unwanted impurities from the sodium hydroxide phase.

15. Discard the chloroform washes. Transfer the sodium hydroxide extracts to a 250-milliliter beaker. Rinse each separatory funnel with two 5-milliliter portions of water and add to the sodium hydroxide extract. Wash each funnel twice with tap water and twice with distilled water before next use.

16. Neutralize the washed sodium hydroxide extract to pH 8.0 by dropwise addition of 3*N* phosphoric acid using a pH meter for pH detection.

17. Transfer the pH 8.0 water extract to a 250-milliliter separatory funnel using 10 to 20 milliliters of water for a rinse.

18. Extract the solution with three separate 50-milliliter portions of carbon tetrachloride. The zeranol transfers to the lower carbon tetrachloride phase. Use the same 30-count phase-mixing technique as in step 9 and allow the mixture to stand 5 minutes for phase separation.

19. Composite the carbon tetrachloride extracts.

20. Extract the carbon tetrachloride composite with two 20-milliliter portions of 1*N* sodium hydroxide. Zeranol transfers from carbon tetrachloride to the upper sodium hydroxide phase. After phase mixing, allow the mixture to stand 5 minutes for phase separation.

21. Composite the sodium hydroxide extracts.

22. Wash the extract with two 50-milliliter portions of carbon tetrachloride. Allow the mixture to stand 5 minutes for phase separation. Discard the carbon tetrachloride washes.

23. Transfer the sodium hydroxide extract into a 250-milliliter beaker. Rinse the separatory funnel with two 5-milliliter portions of water and add to the sodium hydroxide extract. Wash each funnel twice with tap water and twice with distilled water before next use. Adjust the sodium hydroxide extract to a pH of 9.5 by dropwise addition of 3*N* phosphoric acid and transfer to a 250-milliliter separatory funnel using 10–20 milliliters of water for a rinse.

24. Extract the pH 9.5 water solution with three separate 30-milliliter portions of anhydrous ethyl ether. Allow the mixture to stand 5 minutes for phase separation. The zeranol transfers to the upper ether phase.

25. Composite the ether extracts in a 125-milliliter Erlenmeyer flask.

26. Reduce the volume of ether to about 1–2 milliliters by evaporation on a hot plate with low heat while removing vapor from top of flask by vacuum aspiration.

27. Transfer ether residue to a 1-dram glass vial. Rinse down flask side wall with 1–2 milliliters of fresh ether and transfer to the glass vial.

28. Continue evaporation of ether to 0.1 milliliter.

29. Place vial in a vacuum desiccator and evaporate residue at line vacuum and room temperature overnight to dryness.

30. Close vial with a plastic cap and submit ether residue for preparation of TMS derivative and gas chromatographic assay. *Stopping place.*

F. Gas liquid chromatography.

1. Start the gas chromatography and maintain the following operational conditions:

Carrier gas pressure: 50 p.s.i. at tank.

Carrier gas flow rate: Sufficient to give zeranol derivative peak a retention time of 4–8 minutes.

Electrometer range: 10^2 or 10^1.

Detector temperature: 325° C.

Injection port temperature: 325° C.

Column temperature: 250°–280° C., operate isothermally.

Recorder sensitivity: 1 millivolt.

Recorder chart speed: 1 inch per minute.

Sample size: 1 microliter to 5 microliters as necessary to give desired peak area for quantitative measurement.

Septums: Replace each evening and allow to condition overnight at operational temperature.

Flame assembly: Remove silica ash from the flame assembly each week. The flame assembly is removed; the anode, flame jet, and chimney are cleaned with a nylon bristle brush. Water and acetone are drawn through the jet capillary to remove any foreign material.

2. Add 0.2 milliliter of silating reagent to the sample or to the zeranol standard.

3. Stopper the vial and shake vigorously.

4. Warm the vial at 40°–50° C. for a few minutes, then roll the vial on a horizontal plane to insure that all of the interior surfaces of the vial have been in contact with the reagent.

5. Let vial stand for 4 hours or overnight in a warm area (40° C.) to allow reaction to reach completion.

6. Place vial in a small padded centrifuge tube and centrifuge to settle the precipitate and insure that all the liquid is at the bottom of the vial.

7. Inject 1.0–5.0 microliters of clear solution into the chromatograph. At the beginning of the day's run, make 3–5 injections of a standard to condition the column for that day before taking quantitative data.

8. Run known mixtures at the beginning, middle, and end of the day's run over the concentration range of samples to be analyzed to compensate for day-to-day sensitivity fluctuations and drift. If four or less samples are to be run, calibrating at the beginning and end of the run is sufficient.

VI. Calculations

Area values are obtained on known mixtures and samples by multiplying the net peak height by the peak width at half height or by counting squares. Area values obtained on knowns are plotted versus zeranol concentration. Calibration plots indicate a near linear function in the 0–10 microgram range. Area values obtained on samples are converted directly to microgram quantities using the curve. Control tests demonstrated a 70 percent recovery of zeranol from spiked wet beef liver and muscle necessitating a correction factor.

$$\text{Zeranol, parts per billion} = \frac{\text{Micrograms of zeranol found A1,000}}{\text{W A0.7}}$$

Where:

0.7=Correction factor for 70 percent recovery.

W=Grams of tissue examined.

VII. Recovery Study

A. Fortification of reagent blank.

1. For those using this method for the first time either for recovery study or tissue assay, a solvent blank and solvent fortified with zeranol should be processed through the entire procedure. This preliminary operation will establish whether or not the procedure is free from contamination arising from solvents and glassware and demonstrate the level of recovery of the standard zeranol. Level of recovery should be in the same range as the samples.

2. Transfer 600 milliliters of methanol to a 1-liter beaker. Add 50 milliliters of 2*N* HCl to the methanol and concentrate to 125 milliliters by boiling on a hot plate.

3. Transfer 600 milliliters of methanol to a 1-liter beaker. Add 50 milliliters of 2*N* HCl to the methanol and concentrate to 125 milliliters by boiling on a hot plate. Spike the concentrate with 1.0 milliliter of stock solution D.

4. Assay both samples as described in the procedure beginning extraction step V–E1.

B. Fortification of samples.

1. Transfer 100-gram portions of partially thawed tissues into 250-milliliter homogenizing flasks and set half of them aside to serve as tissue blanks.

2. Add to the remaining samples 1 milliliter of stock solution D to serve as fortified samples to which 20 parts per billion zearalanol have been added.

3. Assay both fortified and unfortified tissue as described in the procedure section beginning with V–C1.

[40 FR 13942, Mar. 27, 1975, as amended at 54 FR 31950, Aug. 3, 1989]

§ 556.770 Zoalene.

Tolerances are established for residues of zoalene (3,5-dinitro-*o*-toluamide) and its metabolite 3-amino-5-nitro-*o*-toluamide in food as follows:

(a) In edible tissues of chickens:

(1) 6 parts per million in uncooked liver and kidney.

(2) 3 parts per million in uncooked muscle tissue.

(3) 2 parts per million in uncooked fat.

(b) In edible tissues of turkeys: 3 parts per million in uncooked muscle tissue and liver.

PART 558—NEW ANIMAL DRUGS FOR USE IN ANIMAL FEEDS

Subpart A—General Provisions

Sec.

Subpart B—Specific New Animal Drugs For Use in Animal Feeds

AUTHORITY: Secs. 512, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b, 371).

SOURCE: 40 FR 13959, Mar. 27, 1975, unless otherwise noted.

Subpart A—General Provisions

§ 558.3 Definitions and general considerations applicable to this part.

(a) Regulations in this part provide for approved uses of drugs and combinations of drugs in animal feeds. Approved combinations of such drugs are specifically identified or incorporated by cross-reference. Unless specifically provided for by the regulations, a combination of two or more drugs is not approved.

(b) The following definitions apply to terms used in this part:

(1) New animal drugs approved for use in animal feed are placed in two categories as follows:

(i) Category I—These drugs require no withdrawal period at the lowest use level in each species for which they are approved.

(ii) Category II—These drugs require a withdrawal period at the lowest use level for at least one species for which they are approved or are regulated on a ''no-residue'' basis or with a ''zero'' tolerance because of a carcinogenic concern regardless whether a withdrawal period is required.